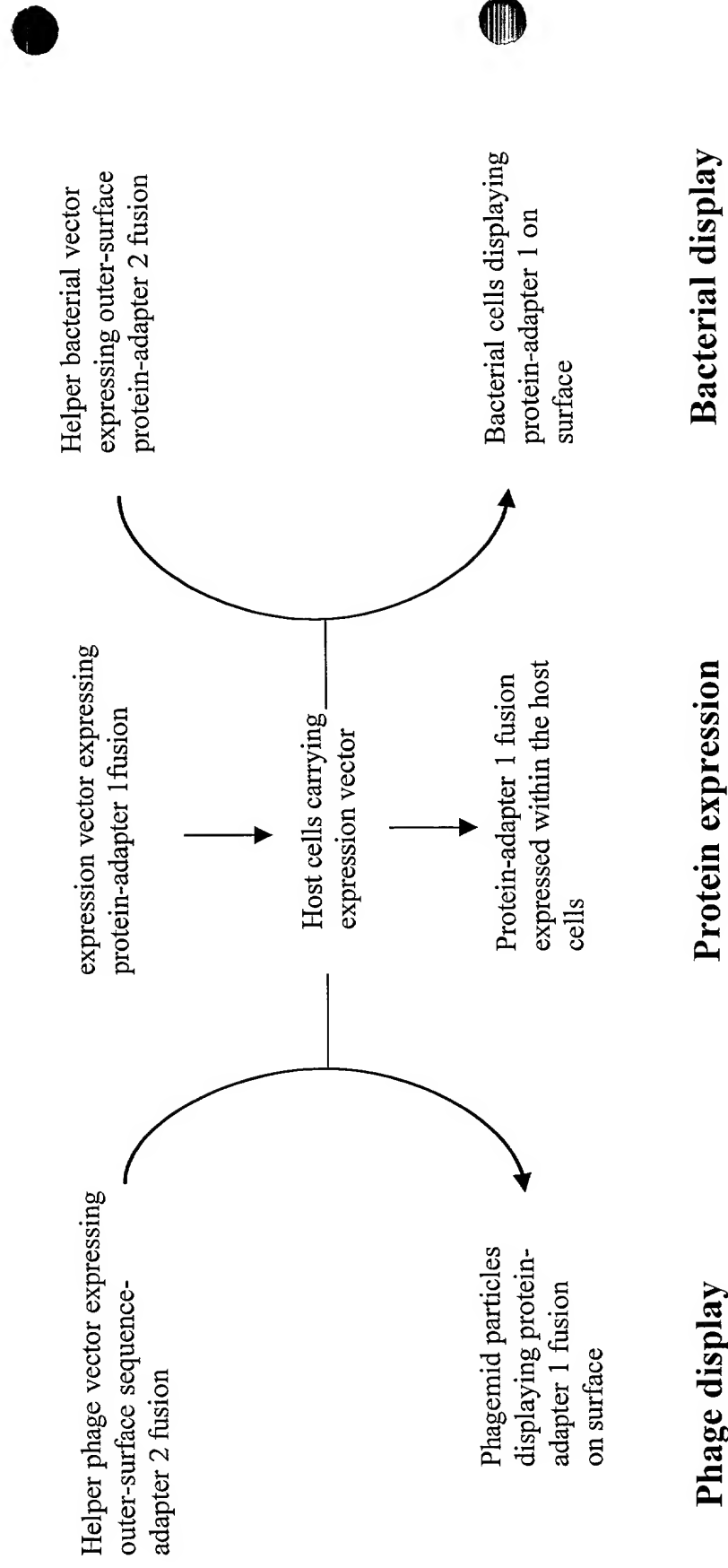


# Adapter-directed display systems



**Fig. 1**

# KO7kpn phage Screening by ELISA

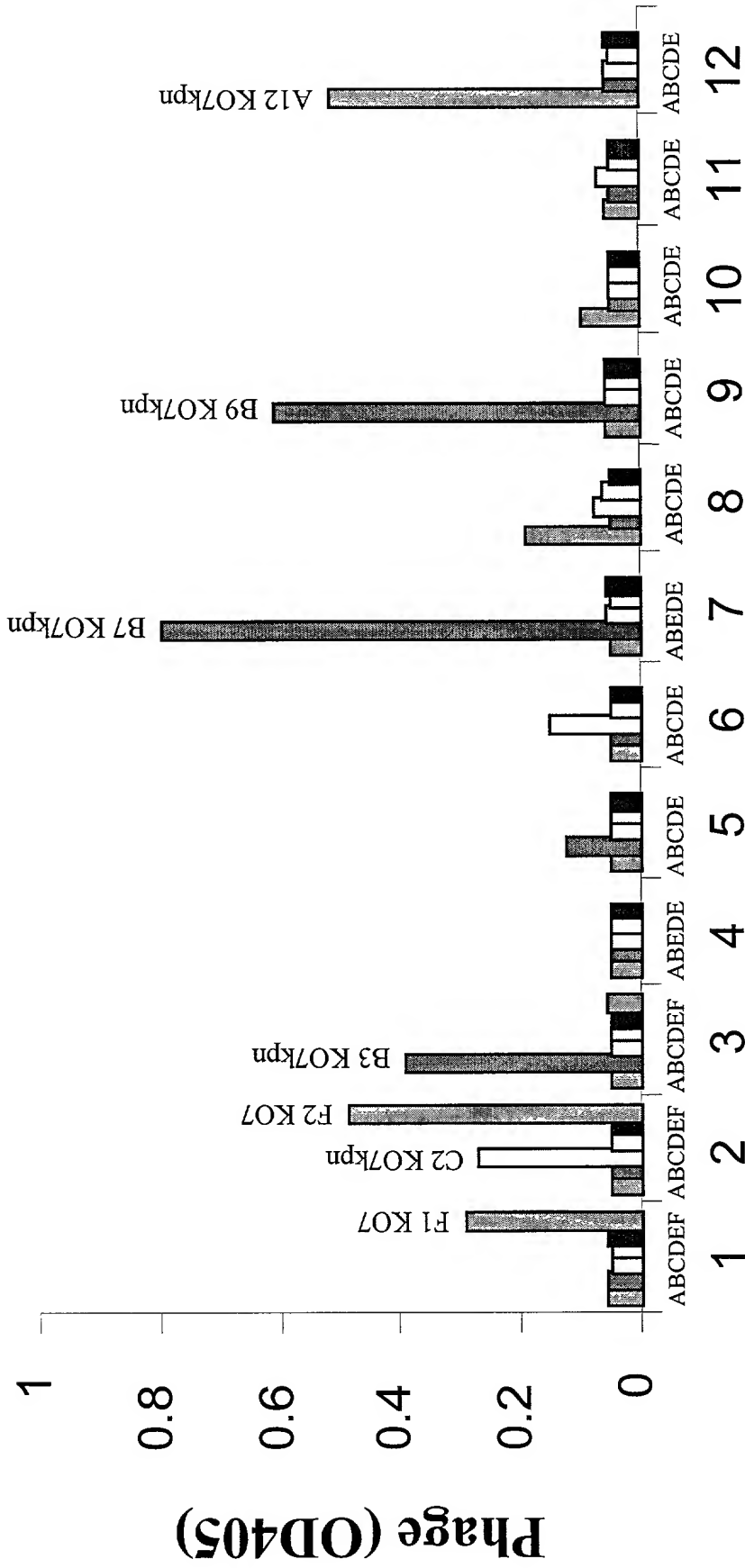


Fig. 2

# KO7kpn helper phage Vector

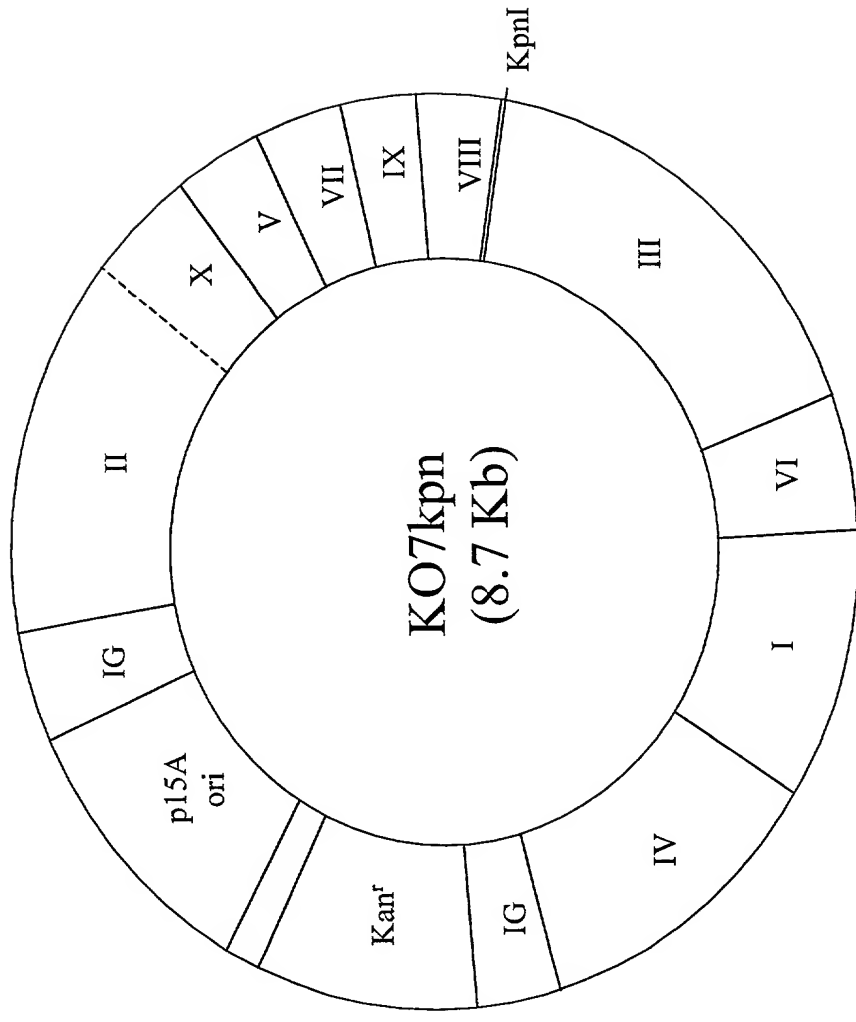


Fig. 3A

Gene III leader sequence in KO7 helper phage

GTG AAA AAA TTA TTA TTC GCA ATT CCT TTA GTT GTT CCT TTC TAT TCT CAC TCC GCT  
V K K L L L F A I P L V V P F Y S H S A

Gene III leader sequence in KO7kpn helper phage

GTG AAA AAA TTA TTA TTC GCA ATT CCT TTA GTG <sup>KpnI</sup> GTA CCT TTC TAT TCT CAC TCC GCT  
V K K L L L F A I P L V V P F Y S H S A

Fig. 3B

## Map of phagemid vector pABMC6

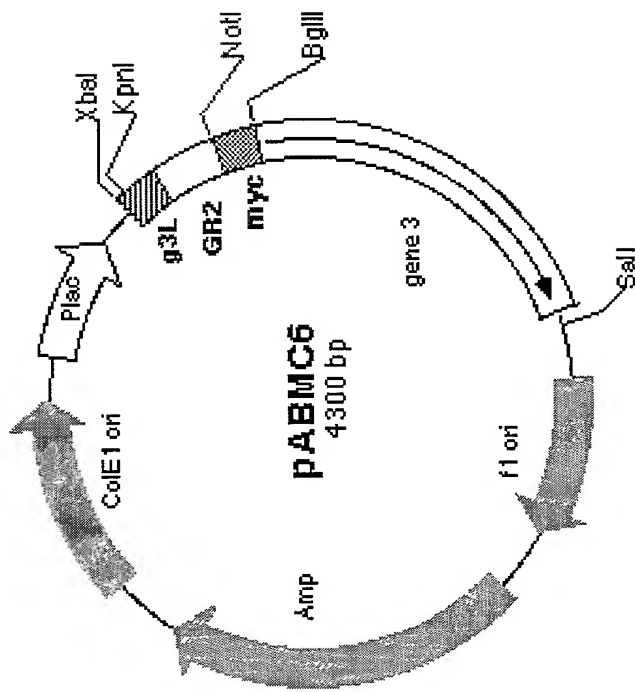
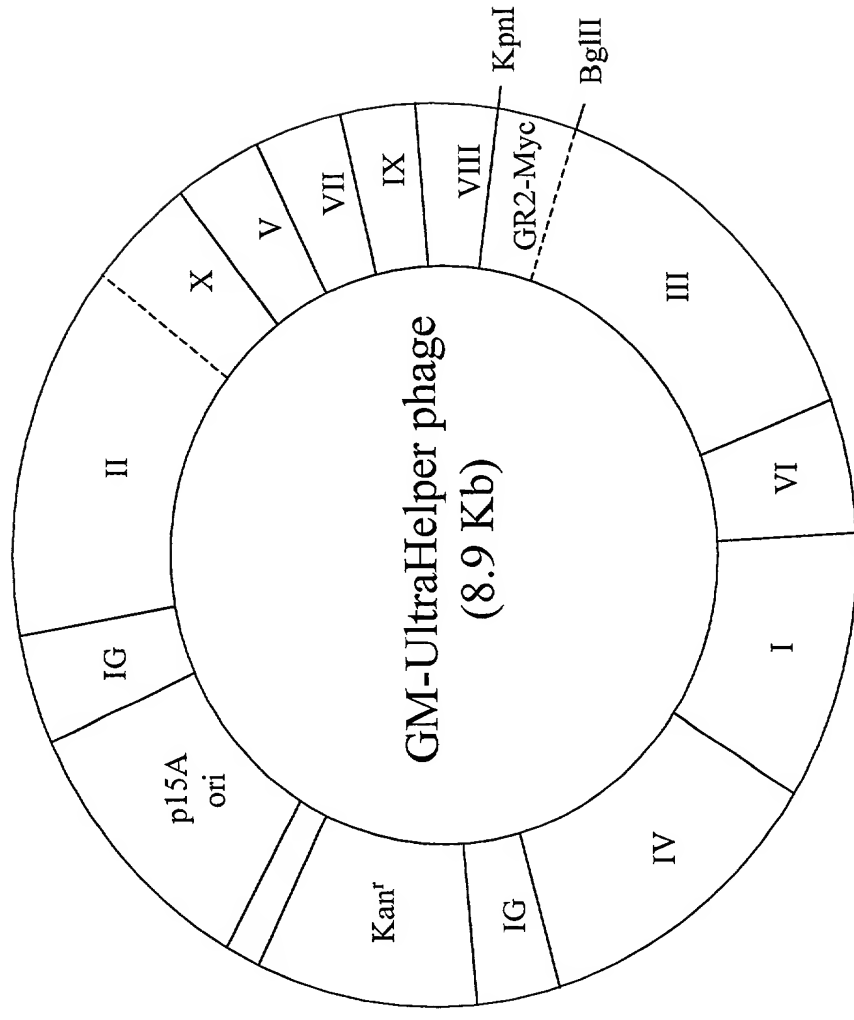


Fig. 4

## Helper phage with engineered gene III fused to adaptor 2



**Fig. 5A**

## GR2-Myc domain coding sequence in GM-UltraHelper phage genome

KpnI	Gene III leader	GR2
---	TTAGTGGTACCTTTCTATTCTCACTCCGCT	ACATCCCGCCTGGAGGCGCTACAGTCAGAAAACCATCGCCTGCGA
-	L V V P F Y S H S A T S R L E G L Q S E N H R L R	
		NotI
	ATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGGTCACCATGCAGCTGCAGGACGTCGGAGGTTGC	GCGGCCGCA
	M K I T E L D K D L E E V T M Q L Q D V G G C A A A	
	Myc-tag	Gene III
	BglII	
	GAACAAAACTCATCTCAGAAGAGGATCTG	AGATCTGGAGGCGGT ACTGTTGAAAGTTGTTTAGCAAAA---
	E Q K L I S E E D L R S G G G T V E S C L A K -	

Fig. 5B

# Trypsin cleavage sites at GR2-Myc domain on GM-UltraHelper phage

## GR2 domain

T S R L E G L Q S E N H R L R M K I T E L D K D L E E V

## *Myc-tag*

T M Q L Q D V G G C A A A E Q K L I S E E D L R S G G G

Fig. 5C





# Detection of GR2-Myc domain on GM-UltraHelper phage

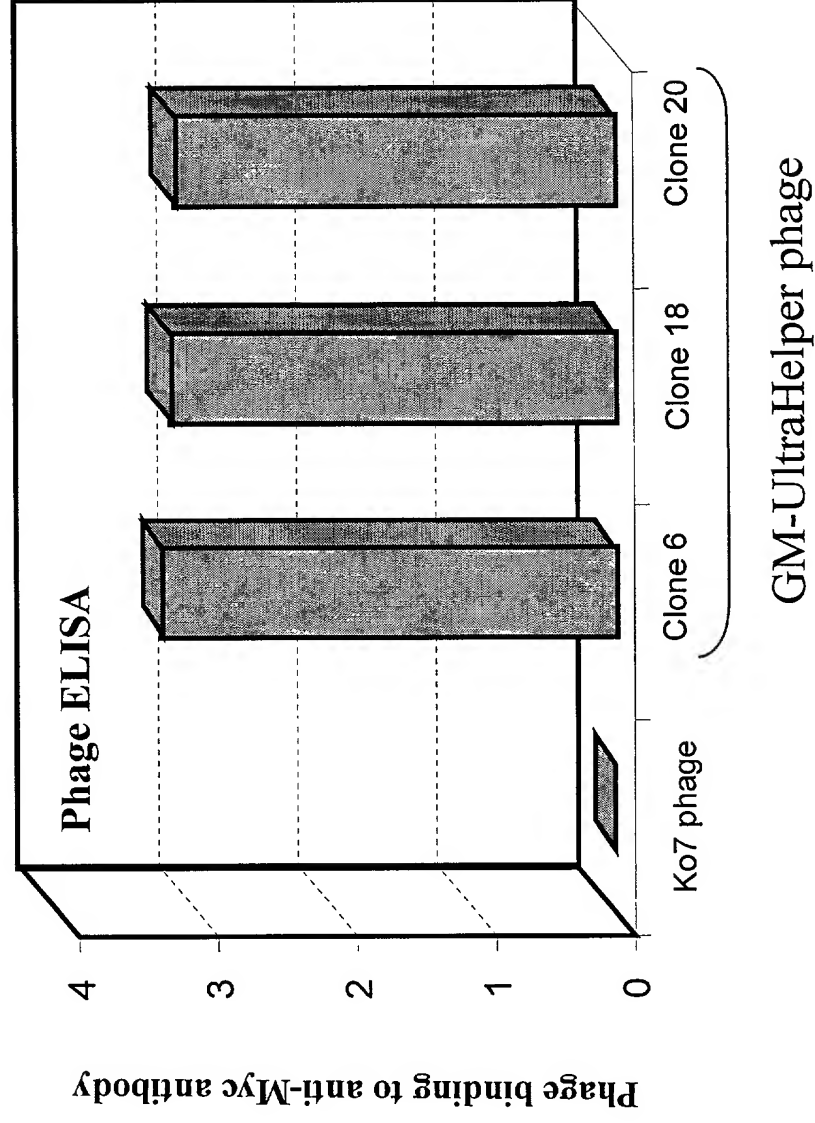
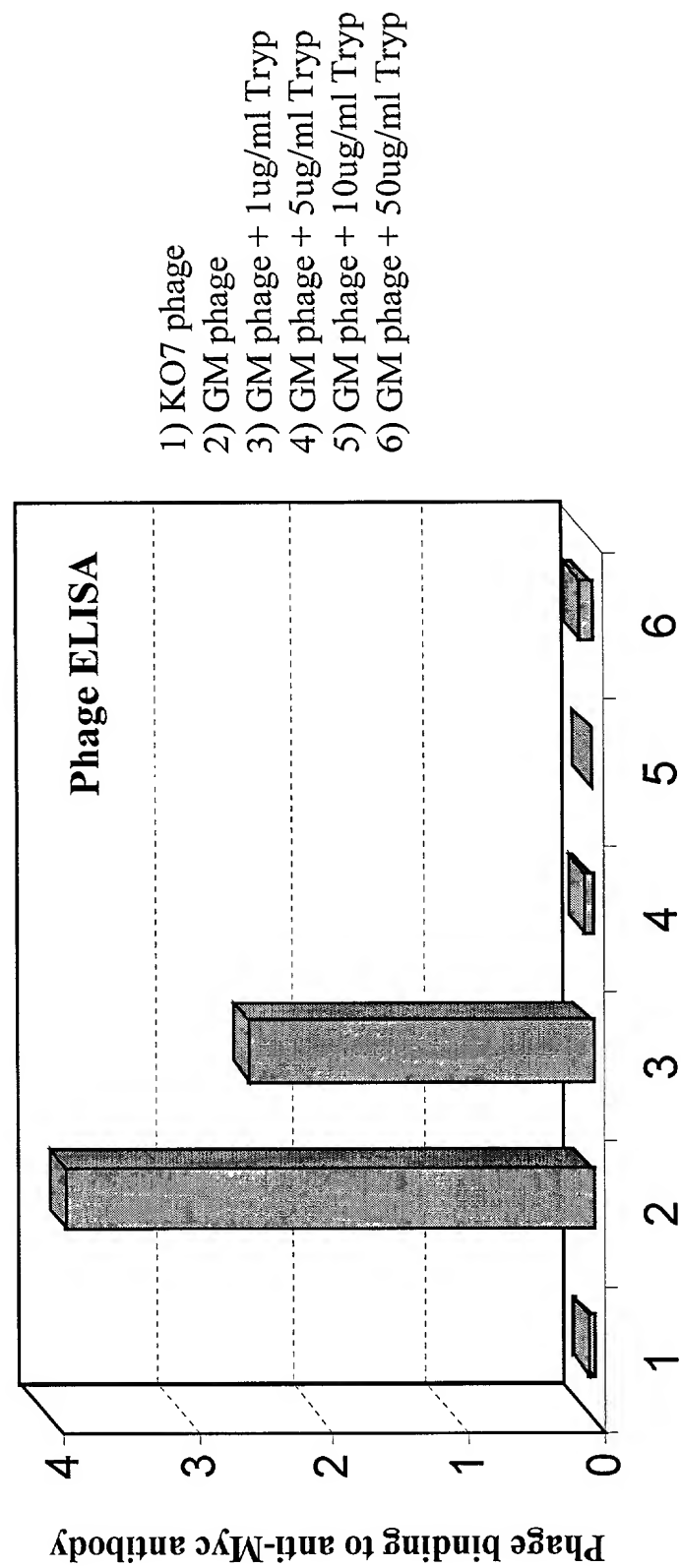


Fig. 7

# Cleavage of GR2-Myc domains on GM phages by trypsin



**Fig. 8**

# Phagemid vector for protein-GR1 expression

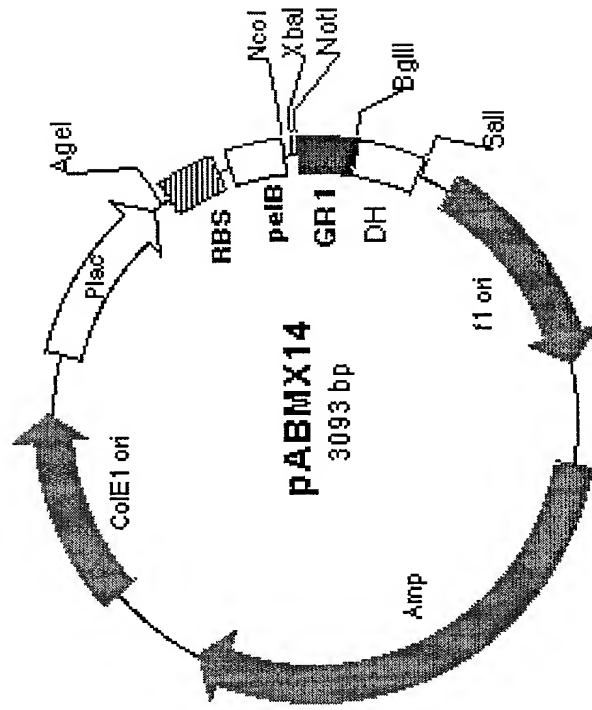
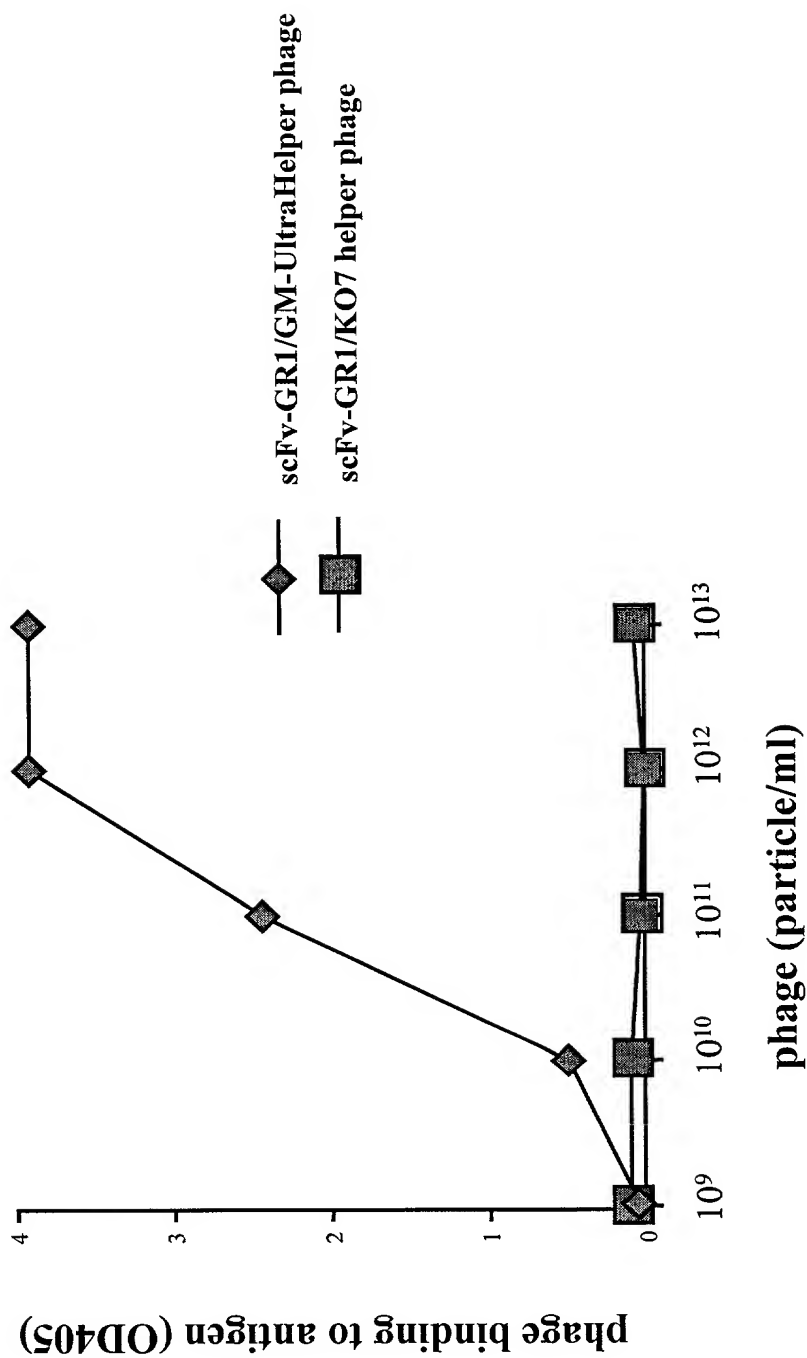


Fig. 9A

Fig. 9B

## Functional display of scFv by GM-UltraHelper phage



**Fig. 10**

## Mutivalent display of scFv by GM-UltraHelper phage

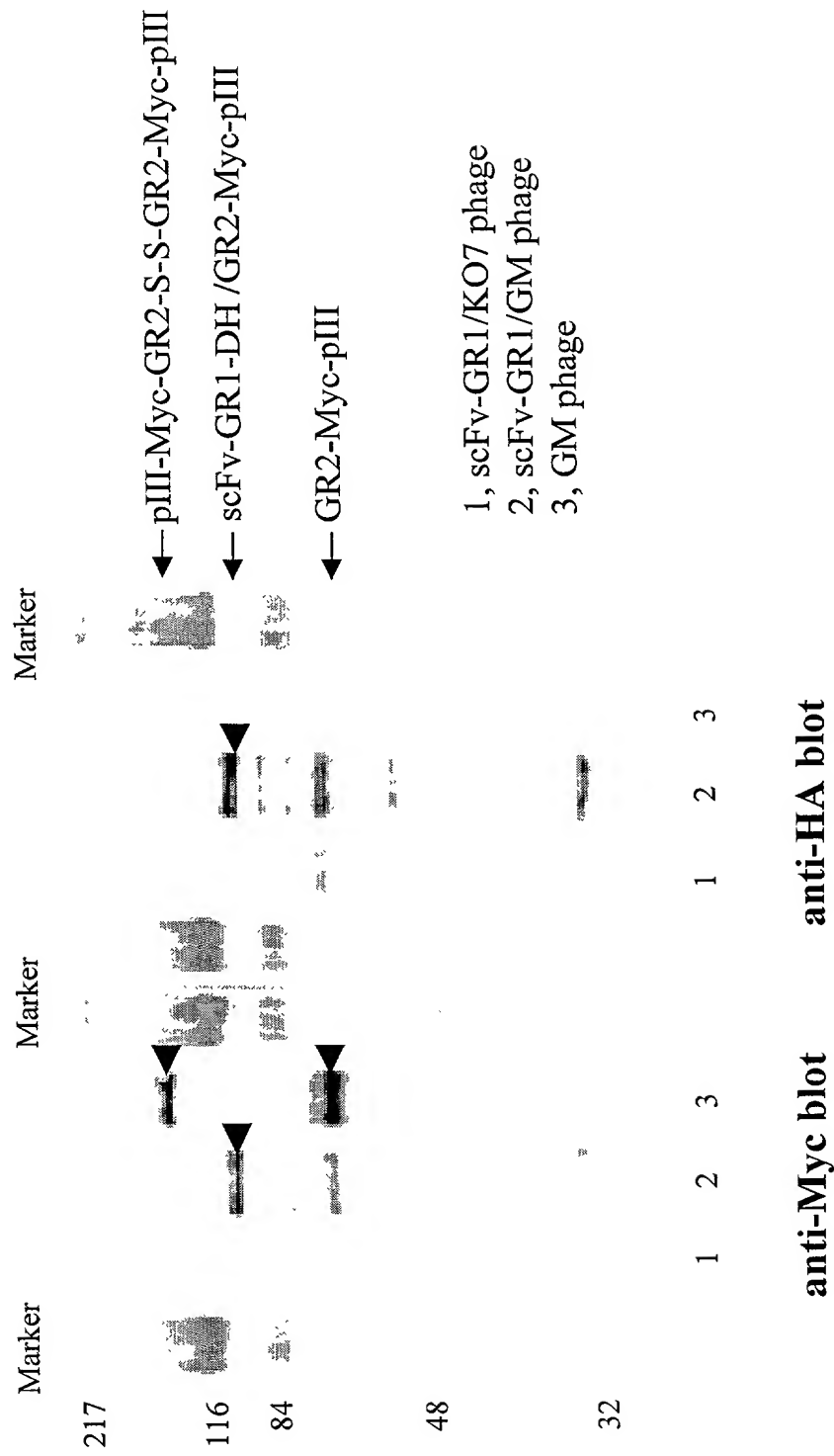


Fig. 11

## Map of phagemid vector pABMC13

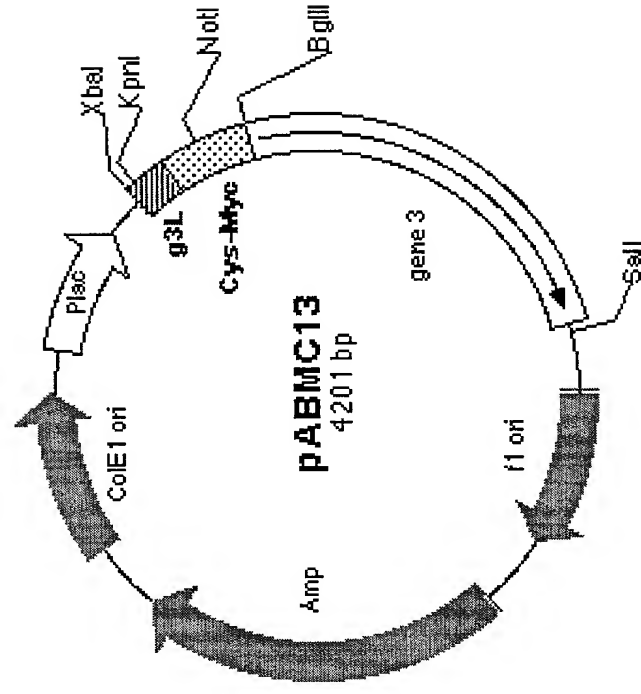
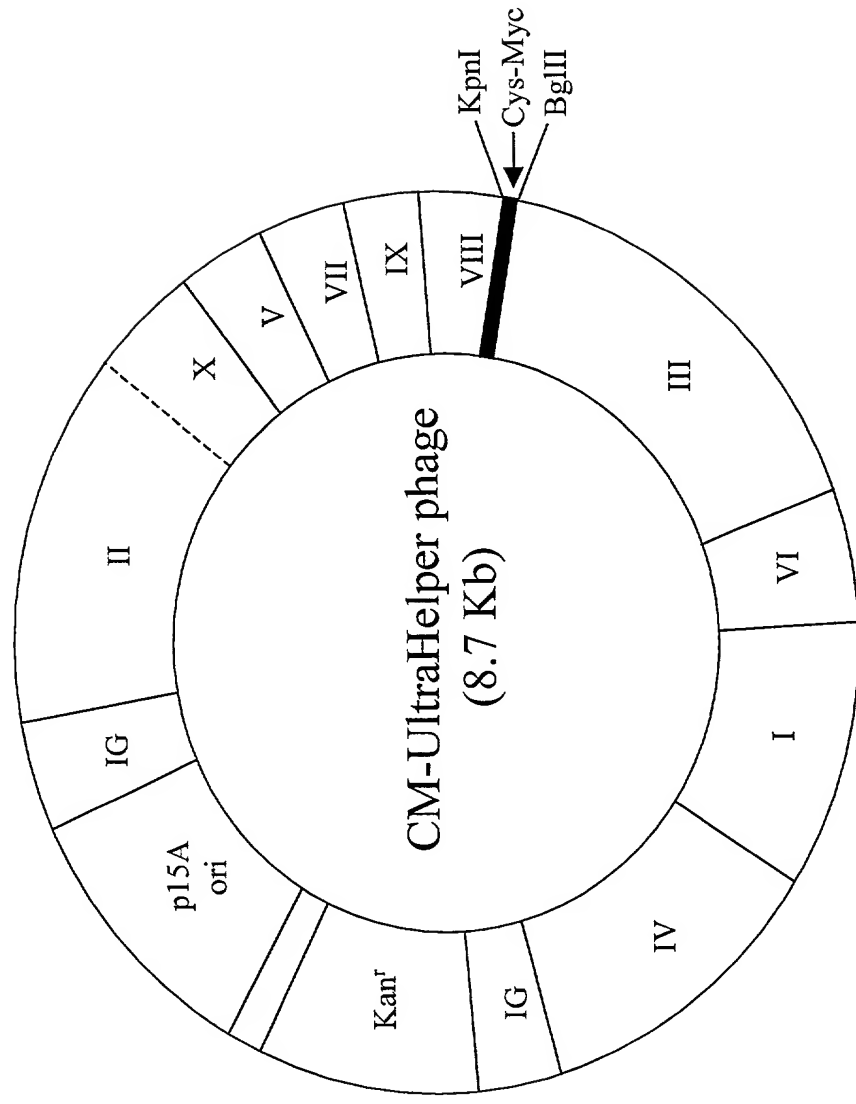


Fig. 12



# Helper phage with Cys-Myc-pIII fusion gene



**Fig. 13A**

Engineered gene III sequence in CM phage

	KpnI	Gene III leader	Amber stop	NotI	Myc-tag	BglII
---	TTAGTGGTACCTTTCTATTCTCACTCCGCT	TAGGCTTGC	GGTGGTGC	CGCGCAGAACAAAACTCATCTCAGAAAGAGGATCTGAGATCT	AGATCTGGA	
-	L V V P F Y S H S A	*	A C G G A A A E Q K L I S E E D L R S R S G			

Gene III

GGCGGT	ACTGTTGAAAGTTGTTTAGCAAAACCTCATACAGAAAATTCATTACTAACGCTCTGGAAGACGACAAAACTTTAGATCGTTACGCT	-----
G G	T V E S C L A K P H T E N S F T N V W K D D K T L D R Y A	- -

Fig. 13B

# Detection of Myc-tag on CM-UltraHelper phages by ELISA

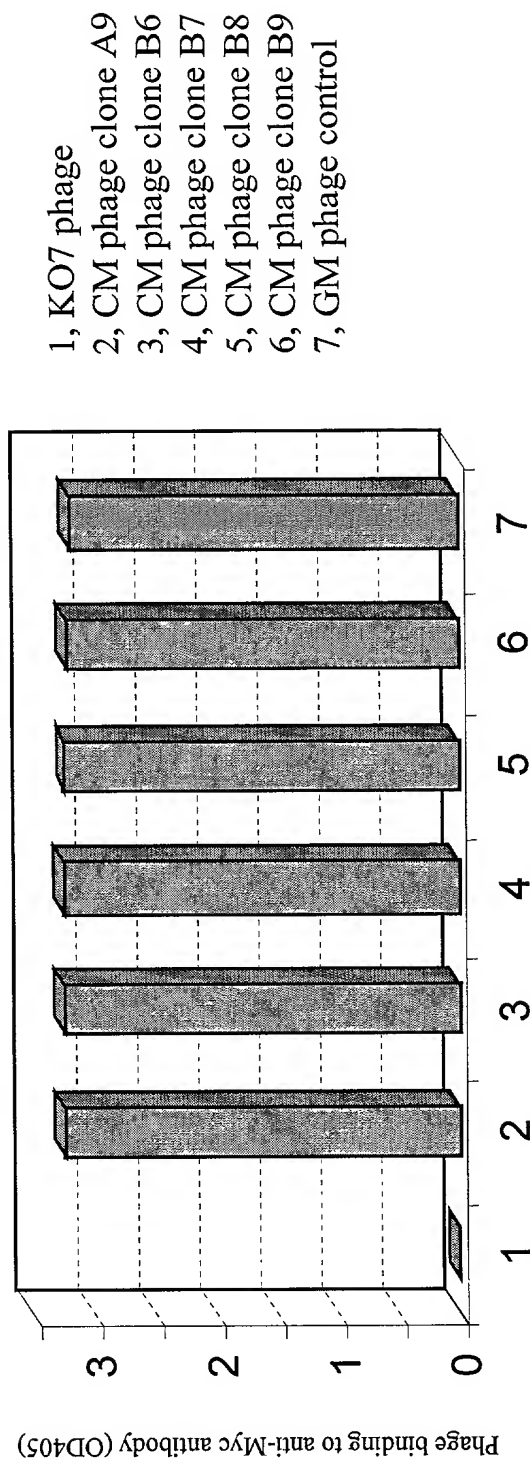
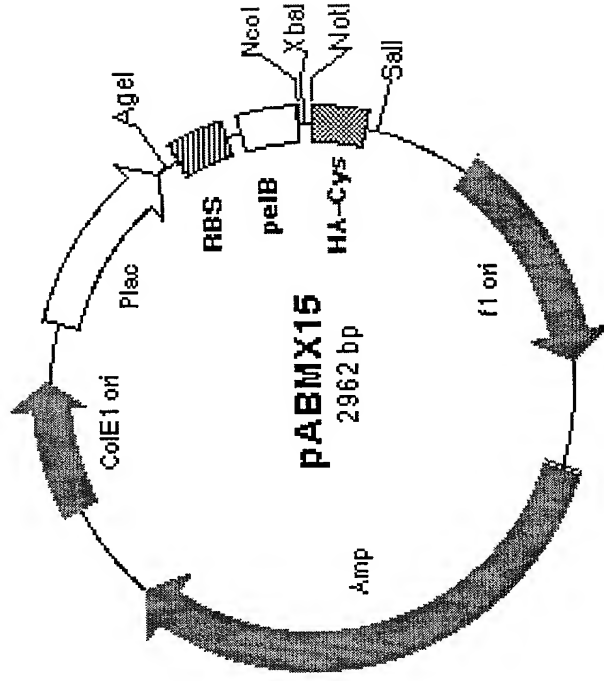


Fig. 14

# Phagemid vector for protein-HA-cys expression



**Fig. 15A**



# Functional display of scFv by CM-UltraHelper phage

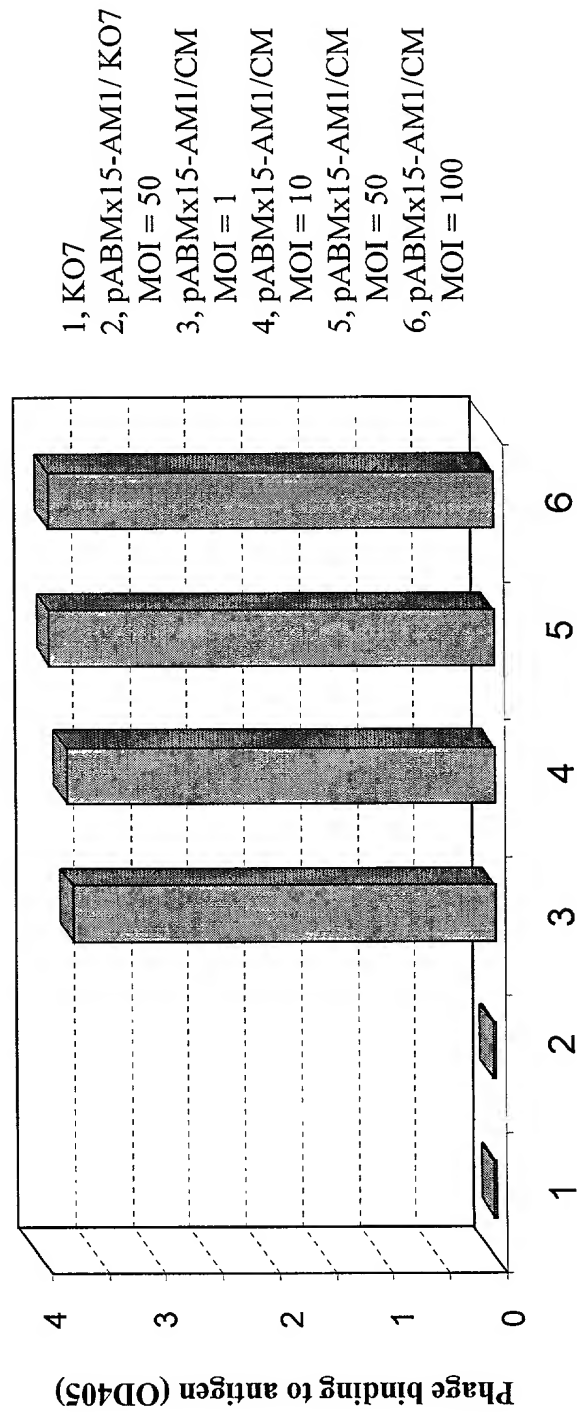
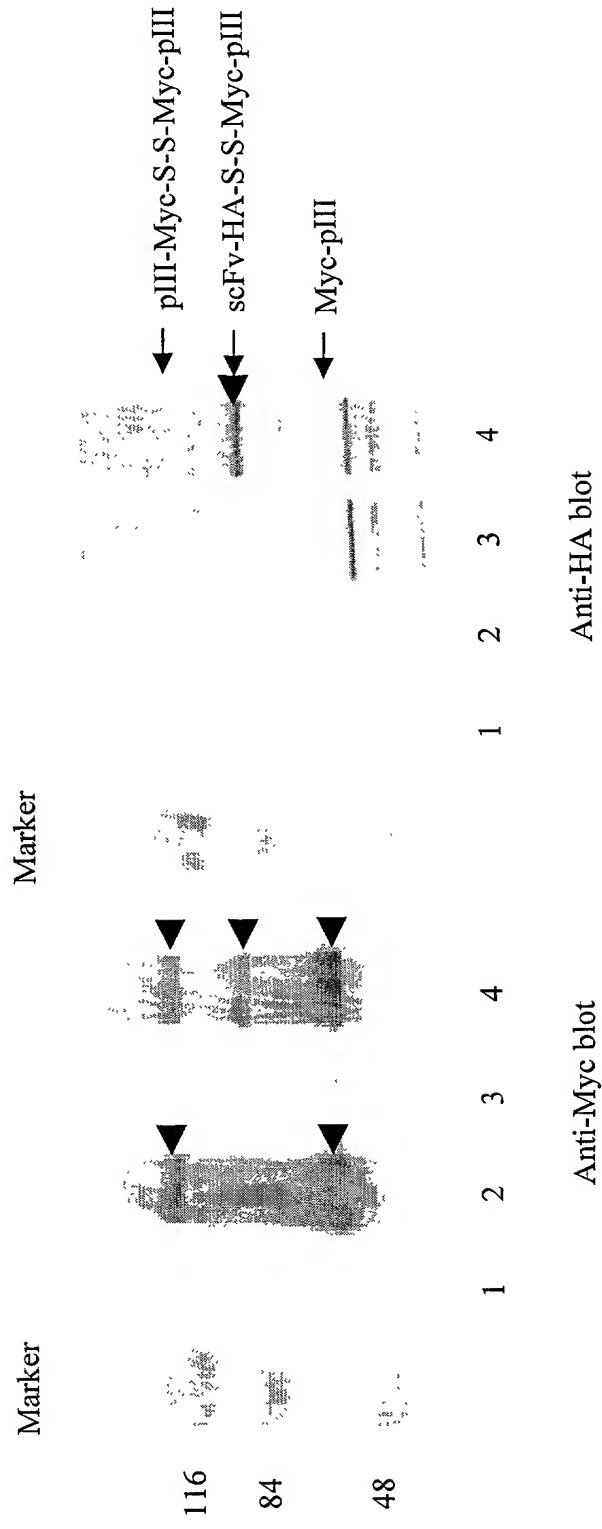


Fig. 16

## Detection of scFv displayed by CM-UltraHelper phage



1: KO7 phage; 2: CM phage; 3: pABMx15-AM1/ KO7; 4: pABMx15-AM1/CM

**Fig. 17**

# Map of phagemid vector pABMC12

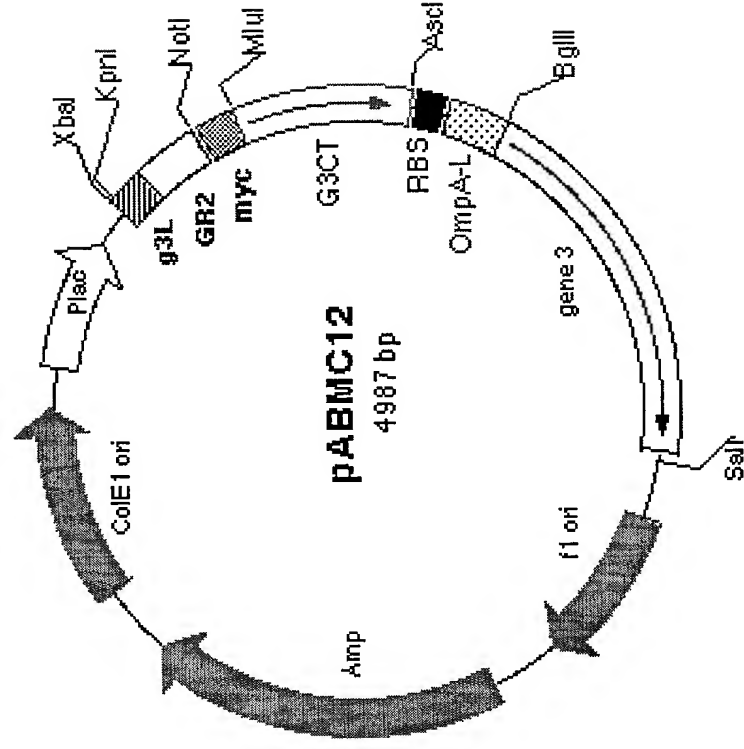


Fig. 18



# Helper phage with an additional copy of engineered gene III

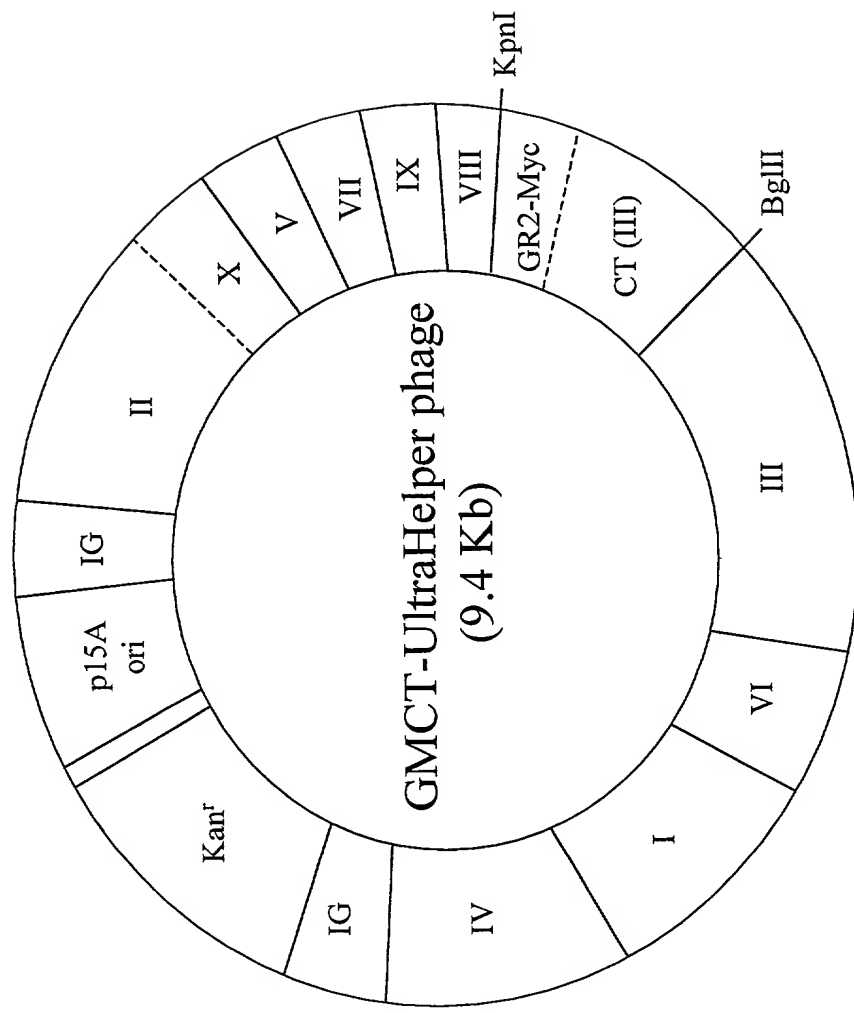


Fig. 19A

# Engineered gene III Sequence in GMCT phage genome

KpnI	Gene III Leader	GR2 domain
--TTAGTGGTACCTTTCTATTCTCACTCCGCT ACATCCCGCCTGGAGGCCCTACAGTCAGAAAAACCATCGCTGCGAATGAAGATCACAGAGCTGGATAAA		
- L V V P F Y S H S A T S R L E G L Q S E N H R L R M K I T E L D K		
		Myc-tag
GACTTGGAAAGGTCACCATGCAGCTGCAGGACGTCGGAGTTGC	NotI	GCGCGCGCAGAACAAAACTGATCTCAGAAAGAGGATCTGCACGCGTGCT GGCGGC
D L E E V T M Q L Q D V G G C A A A E Q K L I S E E D L T R A G G		
CT domain of Gene III		
GGCTCTGGTGGTCTTCTGTGGCGGCTCTGAGGTGGCGGCTCTGAGGTGGCGGCTCTGAGGTGGCGGTTCCGGTGGCGGCTCC		
G S G G G S G G S E G G S E G G S E G G S E G G S E G G S E G G S		
GGTCCGGTGATTTTGATTATGAAAAATGGCAACGCTAATAAGGGGGCTATGACCGAAAAATGCCGATGAAACGCGCTACAGTCTGACGCTAAAGGCAAA		
G S G D F D Y E K M A N A N K G A M T E N A D E N A L Q S D A K G K		
CTTGATTCTGTCGCTACTGATTACGGTGTCTGCTATCGATGGTTTCATTGGTGACGTTTCCGGGCTTGCCTAATGGTAATGGTGCTACTGGTGATTTTGTGGC		
L D S V A T D Y G A A I D G F I G D V S G L A N G N G A T G D F A G		
TCATAATCCCAATGGCTCAAGTCGGTGACGGTGATAATTACCTTTAATGAATAAATTTCCGTCAATAATTTTACCTTCCCTCCCTCAATCGGTTGAATGTCCG		
S N S Q M A Q V G D G D N S P L M N N F R Q Y L P S L P Q S V E C R		
CCTTTTGTCTTTGGCGCTGGTAAACCATATGAATTTTCTATTGATTGTGACAAAAATAAACTTATTCCGTGGTGCTTTTGGGTTTCTTTTATATGTTGCCACC		
P F V F G A G K P Y E F S I D C D K I N L F R G V F A F L L Y V A T	AscI	S/D
TTTTATGTATGTAATTTTCTACGTTTGTCTAACATACTGCGTAATAAGGAGTCTTAATAA	GGCGCGCCACAATTTCAAGTAAGGAGGTTTAATAA	ATGAAA
F M Y V F S T F A N I L R N K E S * *		M K
OmpA leader	BglII	Gene III
AAGACAGCTATTGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTAGCGCAGGCT	AGATCTGGAGGCGGT	ACTGTTGAAAGTTGTTTAGCAAAA---
K T A I A I A V A L A G F A T V A Q A R S G G G T V E S C L A K -		

Fig. 19B

# Functional display of scFv by GMCT-UltraHelper phage

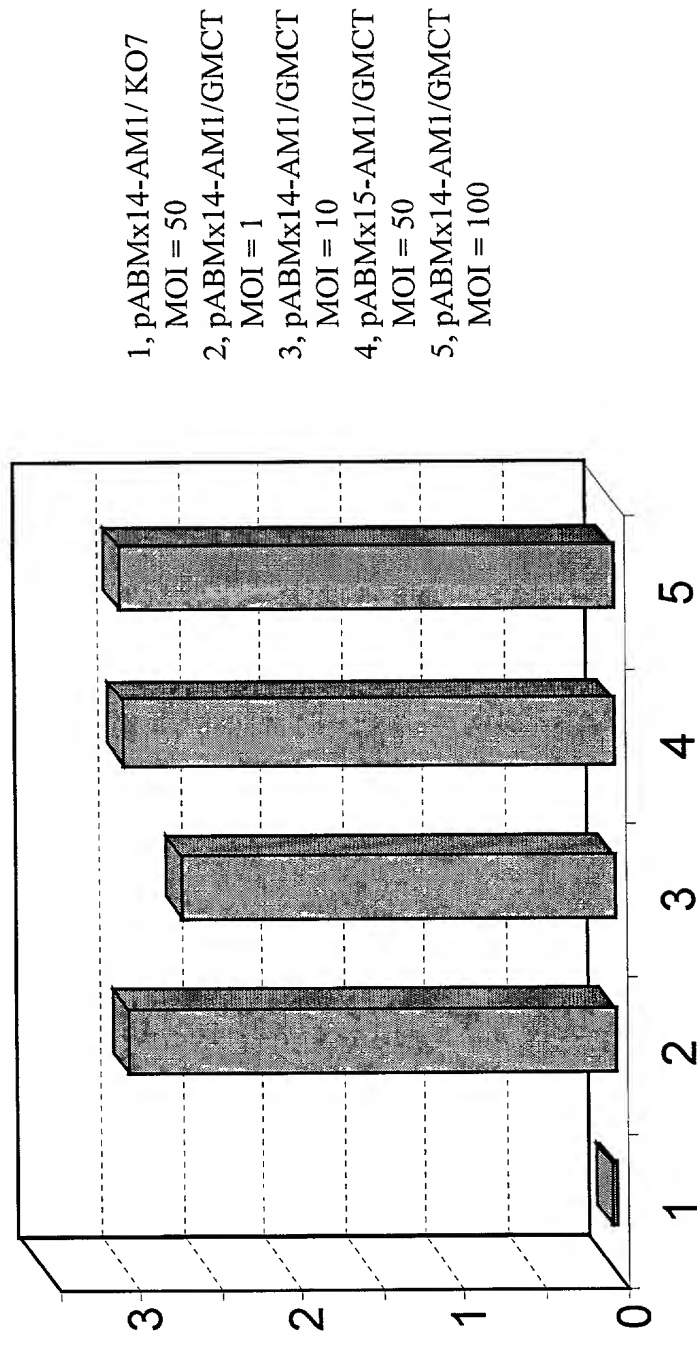
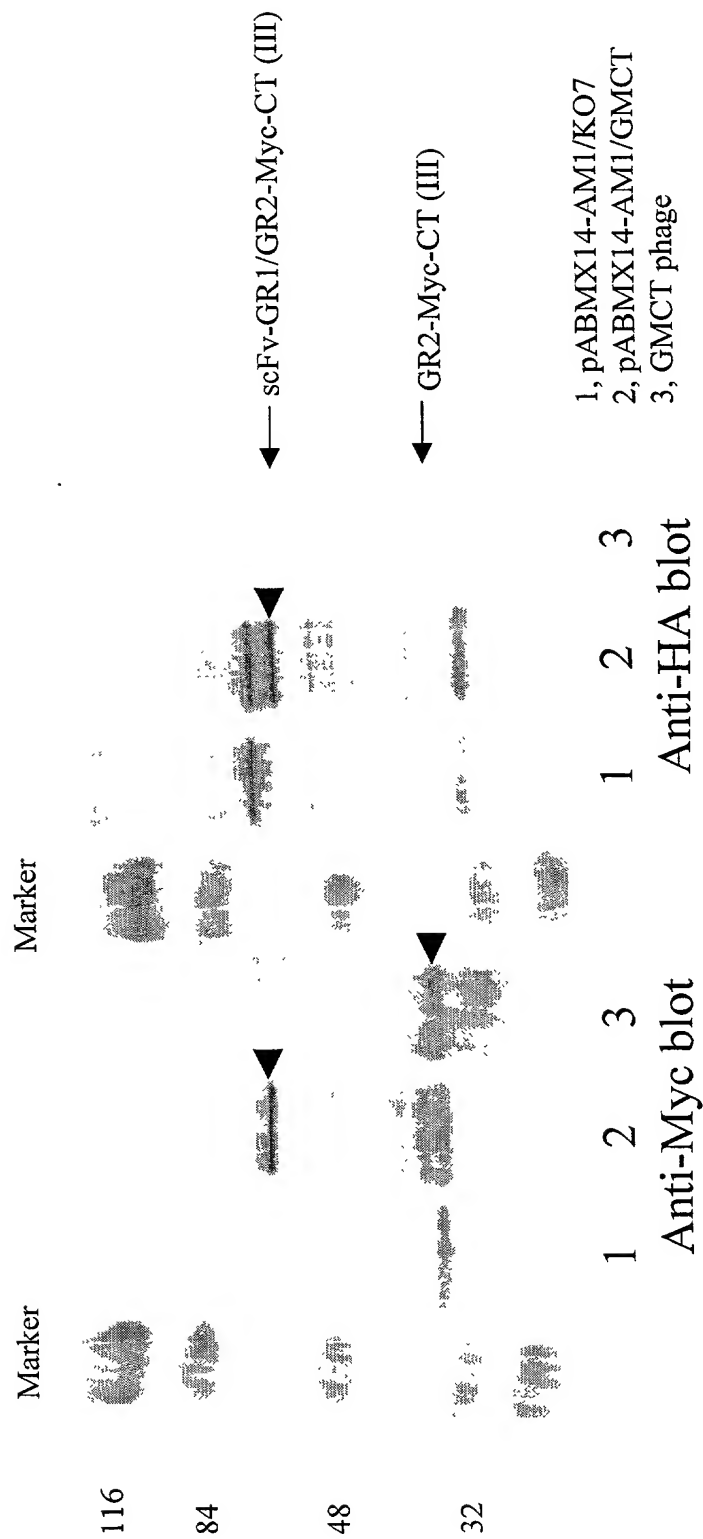


Fig. 20

## Detection of scFv displayed by GMCT-UltraHelper phage



**Fig. 21**

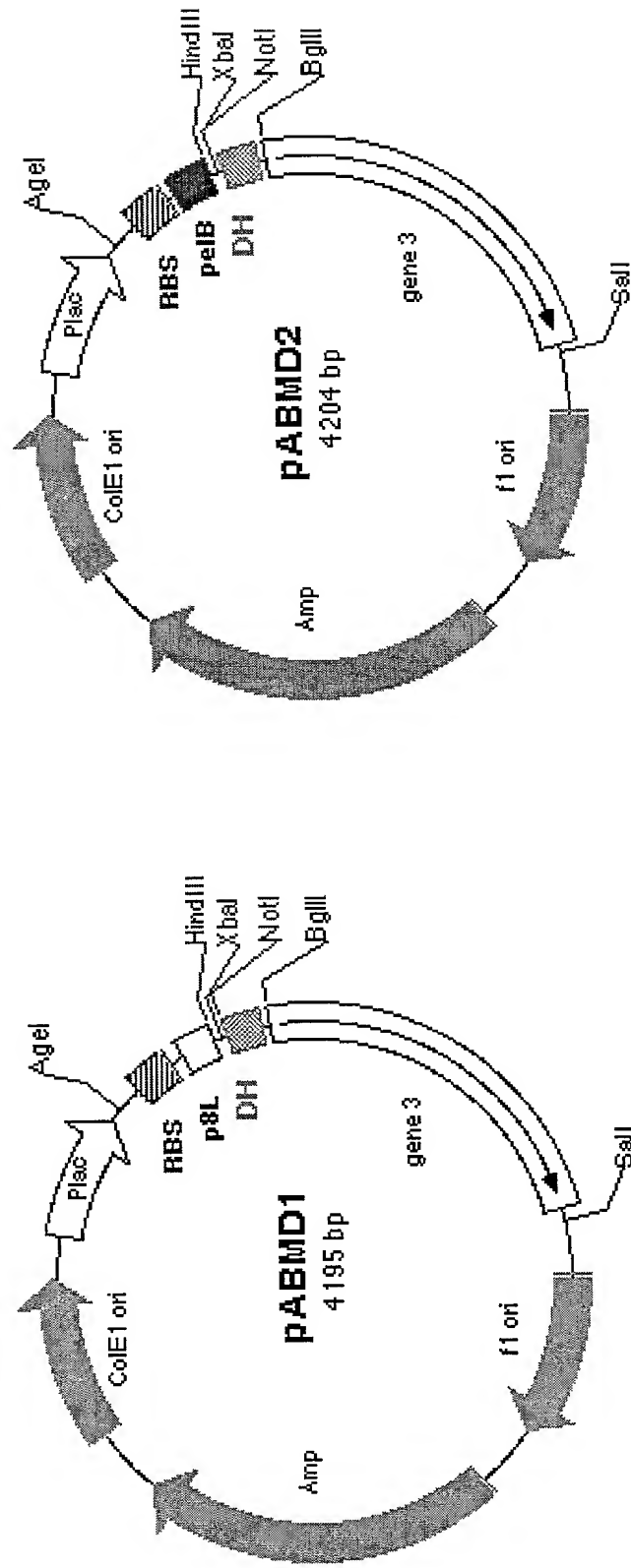


Fig. 22A

# **PABMD1 vector: sequence from AgeI to Sall**

lac promoter/lac O1      AgeI      EP      S/D  
AATTGTGAGCGGATAACAATT ACCGGT TCTT TTAACCTTTAG TAAGGAGG AATTAAAAA  
P8 Leader      HindIII      XbaI  
ATGAAAAAGTCTTTAGTCTCAAAGCCTCCGTAGCCGTTGCTACCTCGTTCCGATGCTAAGCTTCGCT TCTAGA  
M K K S L V L K A S V A V A T L V P M L S F A S R  
NotI      HA-tag      His-tag      Amber stop      BglII  
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT  
A A A Y P Y D V P D Y A G G H H H H H \* R S  
Gene 3      Sall  
GGAGGCGGT ACTGTTGAAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC  
G G G T V E S C L A K ---- A N I L R N K E S \*

# **PABMD2 vector: sequence from AgeI to Sall**

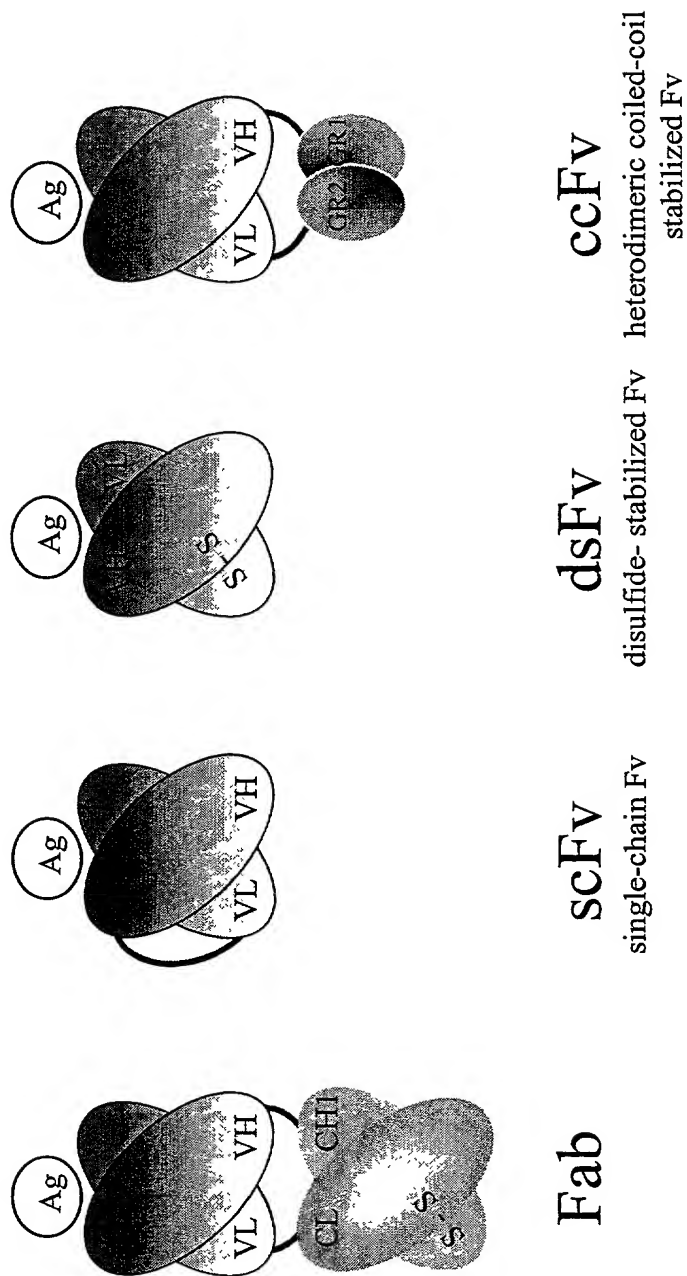
lac promoter/lac O1      AgeI      EP      S/D  
AATTGTGAGCGGATAACAATT ACCGGT TCTT TTAACCTTTAG TAAGGAGG AATTAAAAA  
pelB Leader      Nco I      PstI      XbaI  
ATGAAATACCTATTCCTACGGCAGCCGCTGGATTGTTATTACTCGGGCCAGCCGCCATGGGGCCCTGCAGGCCCTCTAGA  
M K Y L L P T A A A G L L L L A A Q P A M A A L Q A S R  
NotI      HA-tag      His-tag      Amber stop      BglII  
GCGGCCGCT TATCCATACGACGTACCAGACTACGCA GGAGGT CATCACCATCATCACCAT TAG AGATCT  
A A A Y P Y D V P D Y A G G H H H H H H \* R S  
Gene 3      Sall  
GGAGGCGGT ACTGTTGAAAAGTTGTTTAGCAAAA ---- GCTAACATACTGCGTAATAAGGAGTCTTAA GTCGAC  
G G G T V E S C L A K ---- A N I L R N K E S \*

**Fig. 22B**



XbaI 10 20 30 40 50  
TCTAGAGGTGGAGGAGGTGAGGAGAA  
S R G G G G E E K S R L L E K E N  
60 70 80 90 100  
CCGTGAACTGGAAAAGATCATTCGTGAGAAAAGAGGCGTGTCTCTGAAC  
R E L E K I I A E K E E R V S E  
110 120 130 140 AscI  
TGCGCCATCAACTCCAGTCTGTAGGAGTTGTTAATAGGCGCGCC  
L R H Q L Q S V G G C \* \*

XhoI 10 20 30 40 50  
TCTCGAGGAGGTGTTGGAACATCCCGCCTGGAGGCCCTACAGTCAGAAAA  
S R G G G G T S R L E G L Q S E N 100  
60 70 80 90  
CCATCGCCTGCGAATGAAGATCACAGAGCTGGATAAAGACTTGGAAGAGG  
H R L R M K I T E L D K D L E E  
110 120 130 NotI 140  
TCACCATGCAGCTGCAGGACGTCGGAGGTTGCCGCGGCCGC  
V T M Q L Q Q D V G G C A A A

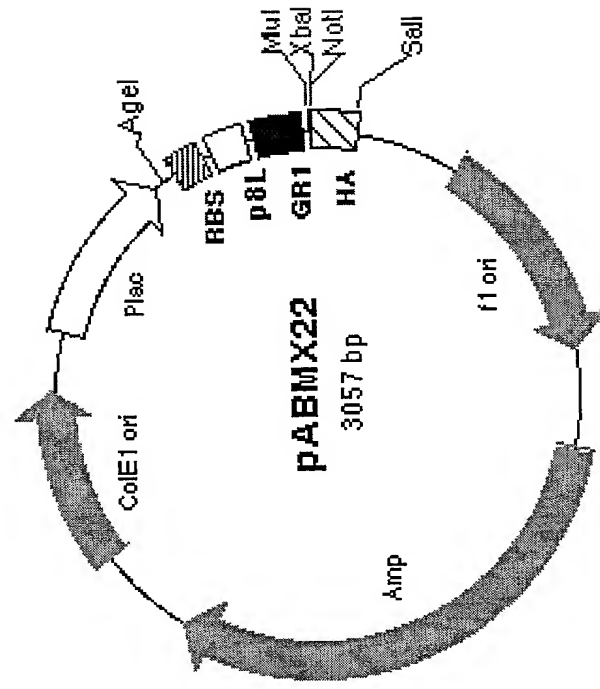


Ag antigen

Fig. 24



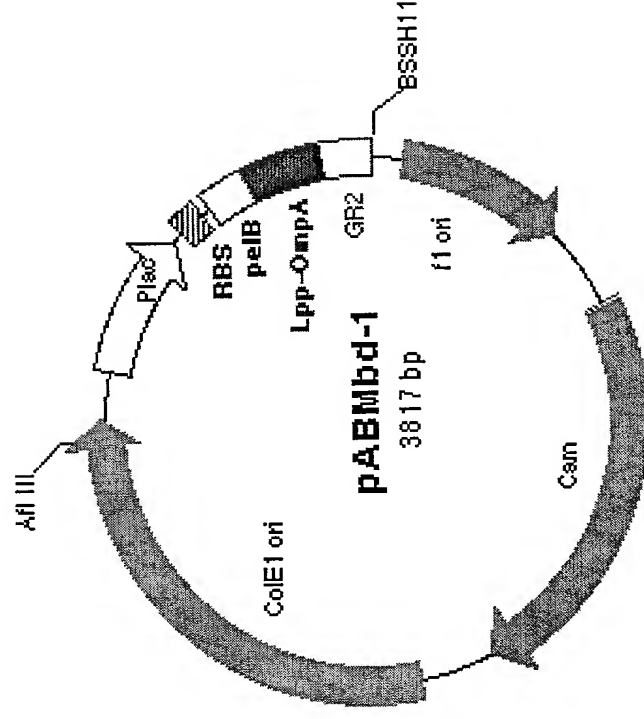
# Expression vector for Adapter-directed bacterial display



**Fig. 25A**



# Helper vector for adapter-directed bacterial display



**Fig. 26A**

